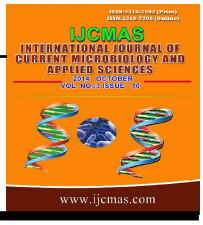
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 783-789**
http://www.ijcmas.com



Original Research Article

Evaluation of α- amylase enzyme from *Bacillus sp.* isolated from various soil samples

M.P.Prasad[1*], Rekha Sethi[2] and M.Anand[1]

[1]Department of Microbiology/Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore 560071, India
[2]Department of Microbiology, Jain University, Bangalore, India
**Corresponding author*

Keywords

Kitchen waste, Sewage waste soil, amylase, SDS-PAGE, *Bacillus spp*.,

A B S T R A C T

Bacterial enzymes are the ones that are having wide variety of industrial applications. Among these enzymes, a-amylase has high demand in industries such as pharmaceuticals, textiles, food processing etc.; so, in this study we have identified bacillus spp., from different source which have high potential to degrade starch. Amylases are digestive enzymes which hydrolyze glycosidic bonds in starch to glucose, maltose, maltotriose and dextrin. They have a lot of potential applications in both food and pharmaceutical industries. The optimisation for the carbon and nitrogen source in the media was carried out to enhance the activity of amylase production. Among all strains studied in this work, E1 showed high protein content as well as comparatively more amylase activity. High Amylase activity of dialysed and crude sample confirms the presence of amylase enzyme in the samples. After Gel filtration of his E1 sample, only Fraction 11 showed high amylase activity which indicates isolation on proteins with amylase activity in that fraction. Thus from results we can conclude that Amylase enzyme was isolated and purified till electrophoresis extent where its molwcular weight was found to be 50-55kDa. Furthur analysis of this purified sample can be done to check its purity and identification of sub types by HPLC and GC. High grade purified sample thus obtained may be used for furthur studies by IR spectroscopy for functional group determination.

Introduction

Microbial enzymes are widely used in industrial processes and α-amylase is one of the most important industrial enzymes, having applications in industrial processes such as brewing, baking, textiles, pharmaceuticals, starch processing, and detergents. α-Amylase are some of the most versatile enzymes in the industrial enzyme sector and account for approximately 25% of the enzyme market (Sidhu GS et al., 1997). α-Amylase catalyzes the endo-hydrolysis of 1, 4-alpha-D-gylcosidiclinkages in polysaccharides

containing 3 or more1, 4- α-linked glucose units. The enzyme acts on starches, glycogen and oligosaccharides in a random manner, liberating reducing groups. Strains of Bacillus have been some of the workhorses of enzyme production for decades, mainly because of their ability to overproduce amylase (Soenshein AL, et al., 1993). *B. Subtilis*, B. *Stearothermophilus*, *B. Licheniformis*, and *B.amyloliquefaciens* are known to be good producers of α-amylase, and they have been widely used for commercial production of the enzyme for various applications (Sivaramakrishnan S,et al., 2006). To obtain maximum yield of an enzyme, development of a suitable medium and culture conditions is obligatory (Narang S, et al., 2001; Ray RC,et al., 2008). Starch or other sugars as a carbon source and ammonium salts or complex organic compounds as a nitrogen source are needed for bacterial growth and enzyme production (Srivastava, et al., 1986; Gangadharan D, et al.,2006). Most amylases are known to be metal ion-dependent enzymes, particularly with regard to divalent ions like Ca2+, Mg2+, Mn2+, and Zn2+(Pandey A, et al., 2000; Polaina J,et al.,2007).

Starch degrading bacteria are most important for industries such as food, fermentation, textile and paper. Thus isolating and manipulating pure culture from various waste materials has manifold importance for various biotechnology industries. In the present investigation a bacterial strain was isolated from soil sample receiving kitchen waste and growth pattern as well as optimum growth condition was determined. Characteristic feature of the strain indicates that it belongs to the genus Bacillus. The optimum temperature for this strain was 37^{o}C, whereas maximum growth was observed at 2% starch concentration. The pH range was found to be 6.8 - 7.2 for optimum growth. Amylase activity was maximum in the temperature range of 50 - 70^{o}C, whereas this temperature range was deleterious for this bacterial strain. Also maximum enzyme activity was observed at 2% of starch concentration. (Sasmita Mishra, et al., 2008).

The purified α-amylase had a molecular mass of 25 kDa in SDS-PAGE. The purified β-galactosidase was a 70 kDa protein. Both the purified enzymes were stable around pHs8-10 and 50°C. α-amylase activity was enhanced by calcium and cobalt, while magnesium andsodium ions inhibited enzyme activity. β-galactosidase was completely inhibited by Ag+, Hg2+ and Cu2+ ions at 1 mM concentration. Mercaptoethanol and SDS inhibited the activity of α-amylase whereas mercaptoethanol restored 80-90% of β - galactosidase activity. The purified enzymes had the capability to hydrolyse starch andlactose when they are immobilized. (D.J. Mukesh Kumar,et al., 2012).

Materials and Methods

Isolataion, identification and screening of microorganisms: Bacillus strain was isolated from soil samples collected from different sites rich in carbohydrates and starch like kitchen wastes, Garden soil and Sewage soil. The bacteria obtained were identified by standard microbiological techniques based on their cell morphology, presence of spores, Gram's staining and biochemical characterization. Pure bacterial isolates were screened for amylase production on starch agar plates.

Optimisation of carbon and nitrogen source: 3 colonies were selected from the soil samples after checking for starch utilisation and biochemical tests, B1 from the sewage sample, E2 from the kitchen soil sample and G26 from garden soil sample.

The amylase production was carried out in 100ml conical flask containing 50ml medium with the respective sources for carbon and nitrogen, incubated with 48hrs in 37^0C followed by inoculation of 24hrs old culture. To study the efficiency of various carbon sources on α-amylase production maltose, dextrose and starch were selected as carbon source. Beef extract, $(NH4)_2SO4$ and peptone were selected as nitrogen sources. The culture broths were centrifuged at 5000 rpm for 20 minutes and the cell free supernatant was used for enzyme determination. Lowry protein assay and DNS assay were carried out to determine the total level of protein and reducing sugar in the culture.

Production and Purification of the enzyme: The E1 sample showed highest reducing sugar content and protein content with Starch and Ammonium sulphate as the carbon and nitrogen source. The amylase produced from the culture was purified by a series of precipitation with 40 and 60 % of Ammonium sulphate, centrifugation and ultra filtration, dialysis and Sephadex 100 column Gel chromatography. Purification was confirmed by sodium dodecyl sulphate (SDS) gel electrophoresis.

Results and Discussion

Screening for amylase activity was carried out on starch agar plate, which was flooded with Gram's iodine to observe the clear zone around the colonies indicating the production of amylase (Figure 3)

Isolataion, identification and screening of microorganisms: The bacterial strain was isolated from kitchen soil, garden soil and sewage soil. A total of 14 organisms were isolated from kitchen soil sample, 8 isolates from sewage soil sample and 11 organisms from garden soil sample (Figure 1(a), (b) and 2).

Production and Purification of Enzyme:

The culture which showed highest zone of clearance on starch agar plate were checked for protein and reducing sugar content by Lowry's and DNSA assay (Figure 4). The optimisation of the microorganisms with different carbon and nitrogen source was carried out and E1 sample from the kitchen waste showed maximum activity with starch and ammonium sulphate as carbon and nitrogen source respectively (Figure 5). Strain B2 shows best activity with Glucose and Peptone.

SDS PAGE : The amylase was produced by E1 isolate in the optimised condition and the protein precipitation was carried out at 40 and 60% ammonium sulphate precipitation, the precipitate were purified by dialysis. The crude samples were then observed using SDS-PAGE. The sample showed 3 prominent bands with molecular weights of 28KDa, 38KDa and 54KDa (Figure 6). The fraction 11 which was separated using sephadex column had bands with 54KDa which determines that the amylase can be extracted with the optimesd conditions and by ammonium sulphate precipitation.

Mohammed Abdu Al-ZaZaee., 2011 produced a-amylase using *Bacillus cereus*, which was purified by DEAE-Cellulose anion exchange and sepharose gel filtration chromatography, resulted in high yield of enhanced purity. Purified alpha amylase was shown approximate molecular weight of about 27 kDa and the optimum activity of amylase was observed at 45^0C.

Yasser R. Abdel-Fattah et al., 2012, investigated that the amylase enzyme

activity was slightly inhibited by detergents, sodium dodecyl sulphate (SDS), or chelating agents such as EDTA and EGTA. On the other hand, great enzyme stability against different divalent metal ions was observed at 0.1 mM concentration, but 10 mM of Cu2+ or Zn2+ reduced the enzyme activity by 25 and 55%, respectively.

Aleena Sumrin, et al., 2011, Purified α-amylase fraction showed a single protein band with a molecular weight of 55 kD. Chemical characterization of the purified α-amylase revealed optimum amylolytic activity at 37°C and pH 7.0 using starch as substrate. It was stable at pH 5.0 to 9.0 and at temperatures 25–70°C.

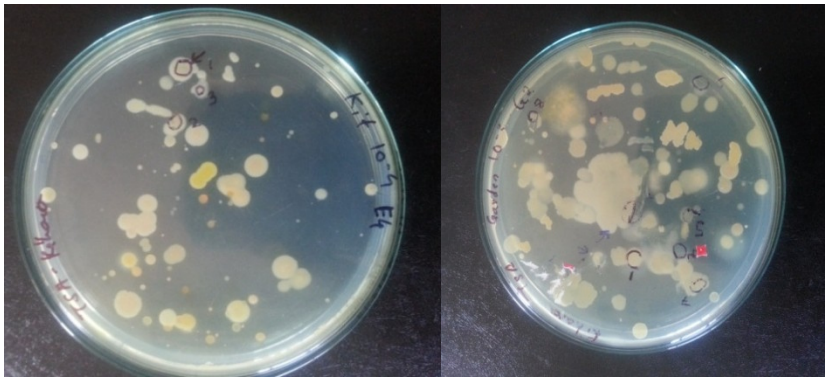


Figure 1 (a) **Figure 1 (b)**

Figure.1 Isolation of microorganisms on Nutrient agar plates from (a) Kitchen waste, (b) sewage soil, (c) garden soil.

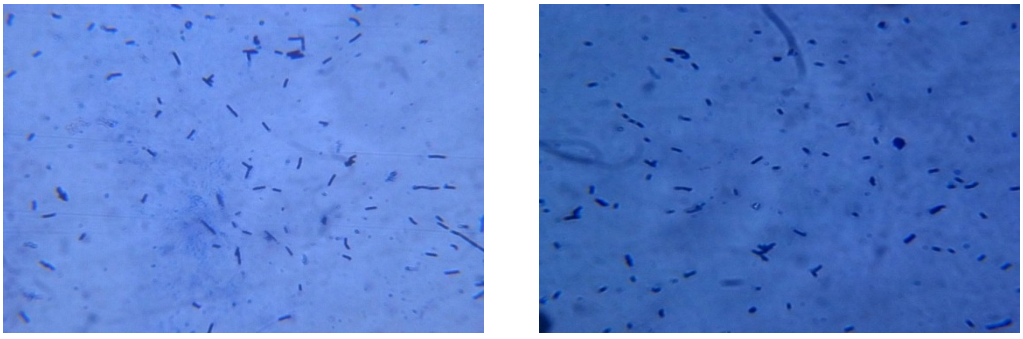
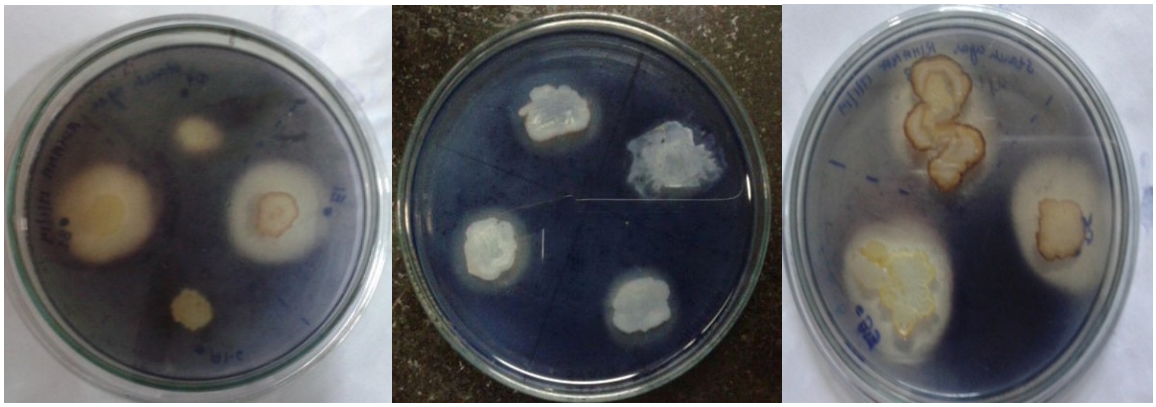


Figure.2 Gram staining Microscopic images of selected strains



A B C

Figure.3 Zone formation on starch agar plate when iodin is added[A:kitchen soil sample, B:sewage soil sample, C:garden soil sample]

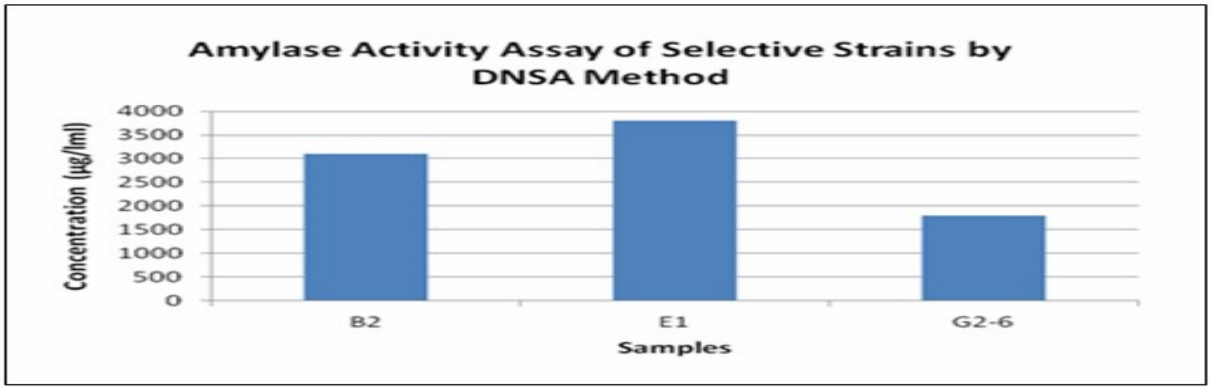


Figure.4 Amylase enzyme assay –DNSA Method

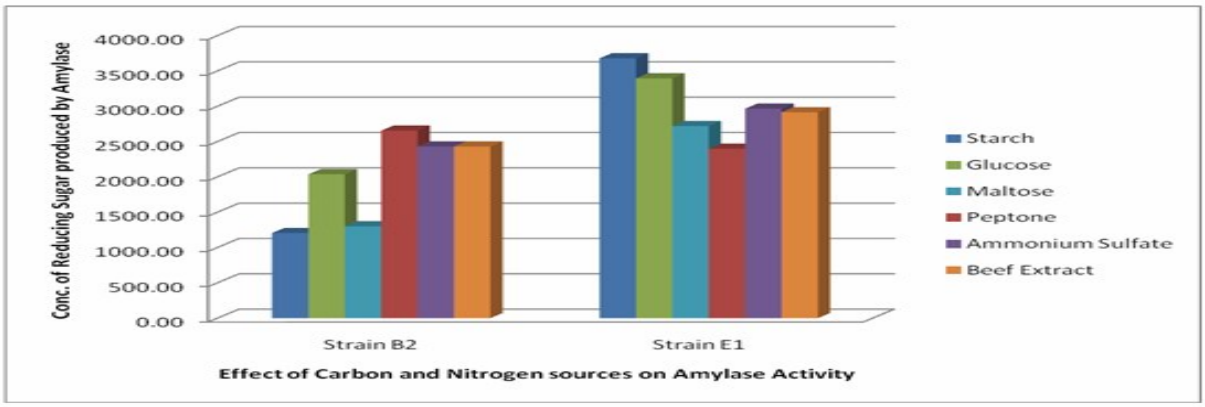


Figure.5 Optimization of Media – Carbon and Nitrogen Sources

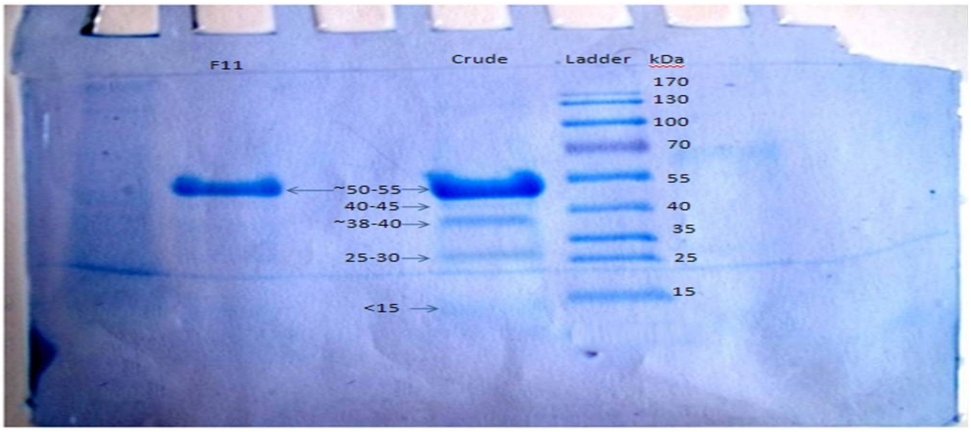


Figure.6 SDS PAGE with CBB staining showing bands for selected samples

References

Aleena Sumrin, Waqar Ahmad, Bushra Ijaz, Muhammad Tahir Sarwar, Sana Gull, Humera Kausar, Imran Shahid, Shah Jahan, Sultan Asad, Mureed Hussain and Sheikh Riazuddin Purification and medium optimization of α-amylasefrom Bacillus subtilis . African Journal of Biotechnology Vol. 10(11), pp. 2119-2129, 14 March, 2011.

D.J. Mukesh Kumar, Jayanthisiddhuraj, B. Amutha, D. Monica Devi, M.D. Bala Kumaran and P.T. Kalaichelvan Purification and Characterization of -Amylase and -Galactosidase from Bacillus Sp. MNJ23 Produced in a Concomitant Medium American-Eurasian J. Agric. & Environ. Sci., 12 (5): 566-573, 2012 ISSN 1818-6769 IDOSI Publications, 2012.

Gangadharan D, Sivaramakrishnan S, Nampoothiri KM et al. culturing of Bacillus amyloliquefaciens for alpha-Amylaseproduction. Food Technol ,Biotechnol 44: 269-274, 2006.

Mohammed Mohammed Abdu Al-ZaZaee, Shivayogeeshwar Neelgund, Gurumurthy D.M andRajeshwara A.N: Identification, Characterization of Novel Halophilic Bacillus Cereus Ms6: a Sourcefor Extra Cellular A-Amylase, Advances in Environmental Biology, 5(5): 992-999, 2011.

Narang S, Satyanarayana T. Thermo stable α-amylase production by an extreme thermophilic Bacillus thermooleovorans. Lett Appl Microbiol 32: 1-35, 2001.

Pandey A, Nigam P, Soccol CR et al. Advances in microbial amylases (review article). Biotechnol Appl Biochem, 31: 135- 152, 2000

Polaina J, MacCabe AP. Industrial enzymes: Structure, Function and Applications. Springer, Dortrecht; 2007.

Ray RC, Kar S, Nayak S et al. Extracellular α-amylase production by Bacillus brevis MTCC 7521. Food Biotechnol 22: 234-246, 2008.

Sasmita Mishra and Niranjan BeheraAmylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes School of Life Sciences,., African Journal of Biotechnology Vol. 7 (18), pp. 3326-3331, 17 September, 2008.

Sidhu GS, Sharma P, Chakrabarti et al. Strain improvement for the

production of a thermo stable α-amylase. Enzyme and Microb Technol 21: 525-530, 1997.

Sivaramakrishnan S, gangadharand, Nampoothiri KM et al.α-Amylases from microbial sources. Food Techno Biotechnol44: 173-184, 2006.

Soenshein AL, Hoch JA, Losick R. Bacillus subtilis and other Gram-positive Bacteria: Biochemistry, Physiology and Molecular Genetics, ASM, New York; 1993.

Srivastava RAK, Baruah JN. Culture conditions for production of thermo stable amylase by Bacillus stearothermophilus. Appl Environ Microbiol 52: 179-184, 1986.

Yasser R. Abdel-Fattah,1 Nadia A. Soliman,1 Nabil M. El-Toukhy,2Hamada El-Gendi,1 and Rania S. Ahmed1, Production, Purification, and characterization of Thermostable α-Amylase Produced by Bacillus licheniformis Isolate AI20 ,2012.